UNIVERSITI PUTRA MALAYSIA

MOLECULAR CHARACTERISTICS AND PATHOGEMCITY OF THE INFECTIOUS BURSAL DISEASE VIRUS ISOLATED IN MALAYSIA

ROOSEVIEN FARIDA NILAWATI BT RACHMAT

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By

ROOSEVIEN FARIDA NILAWATI BT RACHMAT

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of Requirements for the Degree of Master of Veterinary Science

March 2006

DEDICATION

I dedicate this thesis with love and gratitude to my dearest parents, husband and family

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Veterinary Science

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March 2006

Chairman: Associate Professor Mohd Hair Bejo, PhD

Faculty: Veterinary Medicine

Infectious bursal disease (IBD) is an acute viral disease of young chicken and causes serious treat in poultry industry worldwide due to high mortality and immunosuppression. The disease is caused by IBD virus (IBDV), which belongs to the genus *Avibirnavirus* of family *Birnaviridae*. Two distinct serotypes of IBDV are serotype 1 and 2. Serotype 1 is pathogenic to chicken and classified as the classical (ca), very virulent (vv) and variant (va) IBDV. The objectives of this study were to isolate, identify, characterise and determine the pathogenicity of IBDV isolated in Malaysia. IBDV isolates namely UPM0311 and UPM03292 were obtained from field IBD outbreaks in Selangor in 2003. The IBDV isolates were inoculated in specific pathogen free (SPF) embryonated chicken eggs and resulted 100% embryonic mortality within day 3 post inoculation (pi). Acute severe necrotising bursitis was observed in SPF chicken inoculated with the IBDV isolates. The IBDV was detected in lymphoid cells of bursa of Fabricius using immunoperoxidase staining (IPS).

The hypervariable region of VP2 gene of UPM0311 and UPM03292 was amplified by reverse transcriptase polymerase chain reaction (RT-PCR). The sequence were aligned, analysed and subjected to restriction fragment length polymorphism (RFLP). A phylogenetic tree was constructed. The study showed that the UPM0311 and UPM03292 isolates were characterised as vvIBDV and caIBDV, respectively. The nucleotide sequence of UPM03292 and UPM0311 IBDV isolates were submitted to Genbank with the accession number of DQ074690 and DQ074691, respectively. The UPM0311 shared the same amino acid molecular marker for vvIBDV at positions 222(A), 242(I), 253(Q), 256(I), 284(A) and 294(I) of the hypervariable region of VP2. Meanwhile UPM03292 has amino acid substitutions at (A222P), (I242V), (I256V), and (I294L) and unique for caIBDV. The nucleotide sequence for UPM0311 and UPM03292 IBDV isolates were successfully cut by restriction enzyme at *BspMI, Ssp I, Sty I, Taq I* and *BstNI, StyI, SacI, MboI,* respectively. UPM0311 showed highest homologous similarity in nucleotide and amino acid to the reported Malaysian vvIBDV. However, UPM03292 have identical with caIBDV STC and highest similarity with classical hot vaccine (Bursavac). The phylogenetic tree showed that the UPM03292 IBDV isolate located in the same group of the caIBDV and formed subranch with American caIBDV (STC) and American classical hot vaccine (Bursavac). Meanwhile UPM0311 IBDV isolate was group together with vvIBDV and has shared a common evolutionary origin with other Malaysian vvIBDV.

The UPM0311 was inoculated into 28-day-old SPF chickens to determine the response of the bursa of Fabricius, bone marrow and blood to the vvIBDV. The chickens were inoculated with the virus titer of $10^{6.2}$ EID_{50} via oral route. The

chickens were sacrificed at various intervals through 14 days of the trial period. Samples of blood, bone marrow and bursa of Fabricius were collected, processed, examined and analysed. The study showed that the pack cell volume (PVC) and thrombocyte decreased at 2 to 5 days pi. In contrast, the basophil, heterophil, monocyte and lymphocytes were increased at 4 to 12 hours, 3 hours to 2 days, 12 hours to 2 days and 15 minutes to 12 hours pi, respectively. However, the total lymphocyte count was decreased at 1 day to 14 pi. Overall leukocyte was increased at 6 to 12 hours and decreased at 3 to 14 days pi. Histologically, acute moderate to severe cellular degeneration and necrosis were observed in bone marrow at day 2 to 5 pi, but the organ recovered at the late stage of infection. Severe acute necrotising bursitis was recorded at day 2 to 5 pi, whilst at the later stage of infection severe chronic bursitis with severe follicular atrophy was observed. By using immunoperoxidase staining (IPS), the virus was detected in the blood, bone marrow and bursa of Fabricius at 6 hours to 5 days pi, 3 to 5 days pi and 1 to 10 days pi, respectively.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan ijazah Master Sains Veterinar

PENCIRIAN MOLEKUL DAN PATOGENISITI ISOLAT VIRUS PENYAKIT BURSA BERJANGKIT DI MALAYSIA

Oleh

ROOSEVIEN FARIDA NILAWATI BT. RACHMAT

Mac 2006

Pengerusi: Profesor Madya Mohd Hair Bejo, PhD

Fakulti: Perubatan Veterinar

Penyakit bursa berjangkit (IBD) ialah penyakit virus yang akut berlaku pada anak ayam dan mengancam industri ternakan ayam akibat dari kadar kematian yang tinggi dan melumpuhkan sistem imuniti ayam. Penyakit ini disebabkan oleh virus bursa berjangkit (IBDV), di dalam genus *Avibirnavirus* dan keluarga *Birnaviridae*. Terdapat dua serotip IBDV iaitu 1 dan 2. Serotip 1 ialah jenis patogenik pada ayam dan terdapat tiga baka iaitu klasikal (ca), amat virulen (vv), dan varian (va). Objektif kajian ialah untuk isolat, mengesan, menciri dan mengenalpasti patogenesiti IBDV isolat dari Malaysia. Isolat UPM0311 and UPM03292 diperolehi dari kawasan wabak IBD di Selangor pada tahun 2003.

Virus tersebut disuntik dalam telur ayam berembrio bebas penyakit khusus (SPF) di mana kematian 100% embrio berlaku pada hari ketiga selepas inokulasi (pi). Penemuan histopatologikal bagi kedua-dua isolat ialah akut bursitis nekrosis teruk. Ujian pewarnaan immunoperoksida (IPS) positif terhadap kehadiran antigen IBDV di dalam sel limfoid bursa Fabricius.

Kawasan pemboleubah-hiper dalam gen VP2 UPM0311 dan UPM03292 diamplifikasi dengan reaksi rantai polimerase-transkripsi berbalik (RT-PCR). Jujukan gen dianalisis untuk menentukan molekul virus. Pokok pilogenetik dibentuk dan jujukan nukleotida juga dianalisa dengan poliforma fragman enzim pembatasan (RFLP). Melalui keadah pencirian molekul ini isolat UPM0311 telah dicirikan sebagai baka vvIBDV dan telah didaftarkan ke dalam bank gen sebagai DQ074691. Manakala UPM03292 pula dicirikan baka caIBDV, juga didaftarkan di dalam bank gen sebagai DQ074690.

Jujukan asid amino dalam isolat UPM0311 adalah dalam kedudukan 222(A), 242(I), 256(I), 284(A) dan 294(I). Manakala UPM03292 berlaku penukaran asid amino di (A222P), (I242V), (I256V) and (I294L) yang mana unik bagi caIBDV. Jujukan nukleotid UPM0311 telah dipotong oleh enzim pembantas *BspMI, Ssp I, Sty I , Taq I* seperti mana dilaporkan pada vvIBDV lain. Manakala UPM03292 dipotong pula oleh enzim pembantas *BstNI, StyI, SacI, MboI,* di mana unik bagi caIBDV. UPM03292 mempunyai identiti yang serupa dengan STC serta peratus homologi yang tinggi dengan baka kurang nyahaktif iaitu vaksin klasikal bursavac. UPM0311 mempunyai peratus homologi yang tinggi dengan kumpulan baka vvIBDV dari Malaysia. Analisis pokok filogenetik menunjukkan bahawa kumpulan baka vvIBDV termasuk UPM0311 terbentuk dalam satu kumpulan, manakala UPM03292 termasuk dalam kumpulan nyahaktif dan klasikal.

Kajian mengenai tindakbalas vvIBDV terhadap bursa Fabricius, darah dan sum-sum tulang telah dijalankan ke atas isolat UPM0311. Virus UPM0311 dengan titer $10^{6.2}EID_{50}$ dinokulasi melalui oral ke atas ayam SPF berumur 28 hari.

Persampelan diambil dalam beberapa jangkawaktu tertentu sehinggalah 14 hari selepas inokulasi (pi). Sampel darah, sum-sum tulang dan bursa Fabricius diambil, diproses, diperiksa dan dianalisa. Hasil kajian menunjukkan nilai sel mampat (PCV) dan trombosit menurun pada hari ke 2 hingga 5 pi. Sebaliknya berlaku peningkatan pada jumlah bilangan basofil (4 hingga 12 jam pi), heterofil (3 jam hingga 2 hari pi), monosit (12 jam hingga 2 hari pi) dan limfosit (15 minit hingga 12 jam pi). Manakala, bilangan limfosit telah menurun pada hari ke 1 hingga 14 pi. Pada keseluruhanya, jumlah keseluruhan leukosit telah meningkat pada 6 hingga 12 jam pi dan menurun pada hari ke 3 hingga 14 pi. Pemeriksaan histologi ialah akut, sederhana ke teruk sel degenerasi dan nekrosis pada sum-sum tulang berlaku pada hari ke 2 dan ke 5 pi, dan diikuti dengan pemulihan sel pada peringkat akhir jangkitan. Akut nekrosis bursitis yang teruk telah direkodkan pada hari ke 2 hingga ke 5 pi, dengan diikuti kronik bursitis serta atrofi follicular pada peringkat akhir jangkitan. Antigen IBDV telah dikesan melalui kaedah IPS pada sum-sum tulang (3 hingga 5 hari pi), darah (6 jam hingga 5 hari pi) dan bursa Fabricius (1 hingga 10 hari pi).

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I certify that an Examination Committee has met on 6th March 2006 to conduct the final examination of Roosevien Farida Nilawati Rachmat on her Master of Veterinary Science thesis entitled "Molecular Characteristics and Pathogenicity of the Infectious Bursal Disease Virus Isolated in Malaysia' in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relavant degree. Members of the Examination Committee are as follows:

ABDUL RANI BAHAMAN, PhD
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Chairman)

DATO' SHEIKH OMAR ABDUL RAHMAN, PhD
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Internal Examiner)

FAUZIAH OTHMAN, PhD
Associate Professor
Faculty of Medicine and Health Science
Universiti Putra Malaysia
(Internal Examiner)

DHANIRAT SANTIVAR, PhD
Professor
Faculty of Veterinary Medicine
Kasetsart University, Thailand
(External Examiner)

HASANAH MOHD. GHAZALI, PhD
Professor/Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 18 MAY 2006

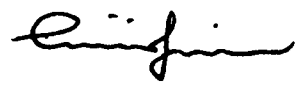
This thesis submitted to the Senate of University Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Veterinary Science. The members of Supervisory Committee are as follows:

MOHD HAIR BEJO, PhD
Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Chairman)

ABDUL RAHMAN OMAR, PhD
Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

AINI IDERIS, PhD
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

RASEDEE ABDULLAH, PhD
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)



AINI IDERIS, PhD
Professor/Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 08 JUN 2006

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledge. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

ROOSEVIEN FARIDA NILAWATI BT. RACHMAT

Date: 17.11.2006

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LIST ABBREVIATIONS

AGPT	Agar gel precipitin test
BALST	Basic local alignment search tool
bp	Basepair
CAM	Chorioallantoic membrane
cDNA	Complementary deoxyribonucleic acid
°C	Degree Celcius
CE	Chicken embryo
CT	Threshold cycle
DEPC	Diethlyl pyrocarbonate
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleoside triphosphate
EDTA	Ethylene diamine tetra acetic acid
EID_{50}	Embryo infective dose fifty
IBD	Infectious bursal disease
IBDV	Infectious bursal disease virus
IFN	Interferon
IPNV	Infectious pancreatic necrosis virus
Kb	Kilobase
M	Molar
$MgSO_4$	Magnesium sulfate
Ml	Mililiter
mM	Milimolar
NaCl	Sodium chloride

NCBI	National Center for Biotechnology Information
NJ	Neighbour-joining
ORF	Open reading frame
PBS	Phospahate buffered saline
PCR	Polymerase chain reaction
pi.	Post infection
Pmol	Picamol
RdRp	RNA dependent RNA polymerase
RT-PCR	Reverse-transcriptase PCR
RT	Room temperature
SPF	Specific pathogen free
TAE	Tris-acetate-EDTA
Tris	2-amini-2-(hydroxymethyl)-1,3propandiol
UPM	Universiti Putra Malaysia
UV	Ultraviolet
Vv	Very virulent
Ca	Classical
Va	Variant
Att	Attenuated

Amino Acid	Single/Three Letter	Amino Acid Code
Alanine	A	Ala
Arginine	R	Arg
Asparagine	N	Asn
Aspartic Acid	D	Asp
Glutamine	Q	Gln
Glutamic Acid	E	Glu
Glycine	G	Gly
Isoleucine	I	Ile
Leucine	L	Leu
Lysine	K	Lys
Methionine	M	Met
Phenylalanine	F	Phe
Proline	P	Pro
Serine	S	Ser
Threonine	T	Thr
Tryptophan	W	Trp
Valine	V	Val